REMARKS/ARGUMENTS

The Invention

The invention is the discovery that a lectin nucleotide phosphohydrolase (LNP) which binds nodulation factors of Rhizobium species, also promotes mycorrhizal associations between the plants and mycorrhizal fungi.

Status of the Claims

Claims 1, 4-5, 9, 11, and 13-14 are pending in this application.

Claims 1, 5, 9, 11, and 13-14 are rejected under 35 U.S.C. §112, first paragraph for allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that the applicant had possession of the claimed invention at the time the application was filed.

Claims 1, 5, 9, 11, and 13-14 are rejected under 35 U.S.C. §112, first paragraph for alleged lack of enablement.

Response to Rejections

In response to the Office Action mailed January 15, 2003, the Applicants submit a declaration under 37 C.F.R §1.132, and the accompanying arguments.

Rejection under 35 U.S.C. §112, first paragraph

Written description

The Examiner rejects claims 1, 5, 9, 11, and 13-14 under 35 U.S.C. §112 for alleged inadequate written description. Applicants respectfully traverse the rejections. The Examiner asserts that the Applicants do not identify structural features unique to the LNP protein, the functional domains of the protein, nor the overall function of the protein and so alleges that the specification contains subject matter that was not described in such a way as to convey that the inventor had possession of the claimed invention at the time the application was filed.

It is well established that "every species in a genus need not be described in order that a genus meet the written description requirement." *Utter v. Hiraga*, 6 USPQ 2d 1709, 1714 (Fed. Cir. 1989). According to MPEP §2163 Sec. II.A.3.(a)(ii), the written description may be met through description of a representative number of species...or by disclosure of relevant identifying characteristics *i.e.*, structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show the applicant was in possession of the claimed genus. The Applicants have clearly met this standard.

In the first paragraph of the present application, Applicants incorporate by reference USSN 09/129,112, filed August 4, 1998. The '112 application is now issued as U.S. Patent No. 6,465,716. The '716 patent discloses characteristic features and functions of the LNP isolated from the legume *Dolichos biflorus*, including that the LNPs of the present invention have carbohydrate binding activity (column 15, lines 40-67, and column 16, 17, 18 and 19, lines 1-16), that LNPs are Nod factor binding proteins (column 16 lines 40-41), and that all of the LNPs of the present invention comprise four conserved sequence motifs characteristic of apyrase enzymes (column 16, lines 57-62). The '716 patent further discloses the isolation of the *Lotus* and *Medicago* LNP proteins using degenerate primers to conserved sequences of *Dochilos biflorus* LNP (column 10, lines 24-37).

The Examiner states that he agrees that the '716 patent discloses carbohydrate binding activity, and teaches the isolation of *Lotus* and *Medicago* LNP proteins. However, the Examiner states that he cannot locate the boxed regions indicating the conserved regions of apyrase proteins in the '716 patent. Furthermore, the Examiner states that the boxed regions indicating conserved domains need to be written in the specification of the present invention.

The Applicants agree that the boxed regions do not appear in the '716 patent. However, these boxed regions, which indicate the conserved domains of apyrase proteins, do appear in the specification of the present invention. Attached to the specification, the original illustration of SEQ ID NO:2 (on page 2, of the attachment) shows the four conserved apyrase domains as boxed regions of the sequence. These conserved motifs are easy to identify in SEQ

ID NOs:4 and 10. Thus, the LNP proteins all share conserved sequence motifs and activity that identify them as an apyrase enzymes.

Adequate written description of the invention may be shown by description of a combination of sufficient, relevant, identifying characteristics (MPEP 2163 II. 3 (a)). The Applicants have elucidated the structural features of LNPs that identify them as apyrase enzymes. They have further elucidated functions unique to LNP apyrases such as carbohydrate binding, that distinguish LNP from other known apyrases. The Applicants have also identified a specific function for the protein in nod factor binding, and in the accompanying declaration under 35 U.S.C. § 1.132, further demonstrate that LNPs function in the establishment of mycorrhizal symbiosis. Therefore, the application describes the claimed invention in sufficient detail to satisfy that one skilled in the art would reasonably conclude that the inventor had possession of the claimed invention at the time the application was filed. The Applicants therefore respectfully request that the rejections for lack of written description be withdrawn.

Enablement

The Examiner rejects claims 1, 5, 9, 11 and 13-14 under 35 U.S.C. §112, first paragraph as allegedly not being enabled for the broad scope of modulating mycorrhizal infection in any plant. Applicant traverses the rejections.

The Examiner alleges that the Applicants have not demonstrated that their invention is sufficient to increase infection when overexpressed by itself in a plant, since there are examples in which overexpressing a protein does not produce the inverse phenotype of the mutant gene. To address this concern, the Applicants direct the Examiner's attention to the accompanying declaration under 37 C.F.R. §1.132, and the parts of the specification noted below.

The Examiner agrees that the assay disclosed in U.S. Patent 6,465,716 would permit one skilled in the art to assay a protein for phosphohydrolase activity, and that the four conserved domains characteristic of apyrase enzymes are most likely sensitive to modification. However, the Examiner alleges that the Applicants have not taught that the specified regions are sufficient for the proper activity of apyrase enzymes involved in mycorrhizal infection.

Furthermore, the Examiner alleges that while the claims are enabled for a method of decreasing mycorrhizal infection of *Lotus japonicus* by *Glomus intraradices* comprising transforming *L. japonicus* with a construct comprising the *L. japonicus* LNP linked in antisense orientation to the CaMV 35S promoter, the specification allegedly does not provide enablement for claims broadly drawn to methods of modulating mycorrhizal infection in any plant.

The Applicants draw the Examiner's attention to the attached declaration under 35 U.S.C. §1.132. Herein, the Applicants show that expression of the *Dolichos biflorus* LNP gene (SEQ ID NO:1) in *Arabidopsis thalania* allows the Arabidopsis to form mycorrhizal associations with *Glomus intraradices* that are otherwise possible for Arabidopsis.

Two transgenic *Arabidopsis* lines DB4 and DB7, were created by transforming wild type *Arabidopsis* with plasmids that express the *Dolichos biflorus* LNP gene (SEQ ID NO:1; paragraph 6). These two transgenic lines, as well as a control strain carring an empty vector, and wild type *Arabidopsis*, were tested for their ability to develop mycorrhizal infections with *Glomus intraradices.*

The seeds of the two transgenic *Arabidopsis* lines that express the *Dolichos biflorus* LNP gene, the empty vector strain, and wild type *Arabidopsis* were added to a soil mixture containing the fungus, *Glomus intraradices*. Sterilized *Lotus japonicus* seeds were placed around the *Arabidopsis* seeds, and all the seeds were germinated. Six weeks later, the *Arabidopsis* roots were collected and stained in order to detect any mycorrhiza (paragraph 7).

The results showed external hyphae attaching to the root surface of the transgenic strains expressing the *Dolichos biflorus* LNP gene, but not attaching to the empty vector strain nor wild type *Arabidopsis* plants. Thus, those plants expressing the transgenic *Dolichos biflorus* LNP gene were able to form mycorrhizal attachments with hyphae of *Glomus intraradices,* whereas the plants that did not express the *Dolichos biflorus* LNP gene did not form these attachments. Therefore, overexpression of LNP in a transgenic plant is sufficient to increase mycorrhizal infection of that plant.

The LNP proteins are related proteins that share structural features and functions and are evolutionarily conserved (Roberts *et al.* (1999) *Mol Gen Genet* 262:261-267, previously submitted). In particular, sequence analysis of these and other genes indicates that the *Dolichos*,

Medicago, and *Lotus* LNP genes are orthologs (*i.e.* are genes that perform the same or similar function). Thus, those of skill would reasonably expect to achieve results similar to those disclosed above when heterologus *Lotus* (SEQ ID NO:10) and *Medicago* (SEQ ID NO:4) LNP genes are expressed in plants. Indeed, during the prosecution of related U.S. Patent application 09/129,112, now U.S. Patent 6,465,716, the Examiner agreed that the *Lotus* (SEQ ID NO:10) and *Medicago* (SEQ ID NO:4) and *Dolichos* (SEQ ID NO:2) LNPs were related, orthologus proteins.

To satisfy the enablement requirement, an application must contain sufficient information regarding the subject matter of the claims so as to enable one skilled in the art to make and use the claimed invention. MPEP §2164.01. The test for enablement is set forth in *In re Wands*, 858 F.2d 731, 8 USPQ 2d 1400 (Fed. Cir. 1988), and requires consideration of multiple factors including: the breadth of the claims; the nature of the invention; the state of the prior art; the level of predictability in the art; the amount of direction provided by the inventor; the existence of working examples; and the quantity of experimentation needed to make or use the invention based on the content of the disclosure.

In the present case, the claims are directed to polypeptides with a defined structure and readily testable activity. The pathways leading to mycorrhizal symbiosis are evolutionarily conserved, therefore the results of experiments with one species make experiments in other species predictable. Although some experimentation may be necessary to distinguish LNP proteins from all proteins with 70% sequence homology to SEQ ID NO:10, such experimentation utilizes well-established techniques, is routinely conducted in the art, and thus does not constitute undue experimentation MPEP §2164.01.

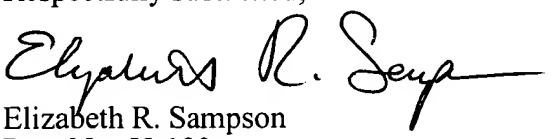
In light of the evidence presented in the accompanying declaration under 35 U.S.C. §1.132, the Examiner must provide reasoning or evidence that one of skill would not be able to practice the claimed invention without undue experimentation. The Applicants therefore respectfully request that the rejections for lack of enablement be withdrawn.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,



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Attachments
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